ON THE BIOLOGY OF DROSOPHILA IMMIGRANS STURTEVANT WITH SPECIAL REFERENCE TO THE GENETIC STRUCTURE OF POPULATIONS[1]

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TAXONOMY

The species which forms the subject of this account was first identified by Sturtevant (1918) as *Drosophila tripunctata* Loew. After re-examination of type material he described it as a new species, *Drosophila immigrans* (Sturtevant, 1921). Duda (1924) splits the Drosophila into a number of subgenera and places this species in the subgenus Spinulophila, subgen. n. He separates Drosophila s. str. from Spinulophila on the basis of a row of short, black bristles on the inner aspect of the first femur in the latter. Sturtevant (1939) in a recent careful study based on the comparison of many species of Drosophila in respect to a large series of external and internal characters has considered a number of Duda's subgenera to be invalid. He proposes dividing the genus into three subgenera. On this basis *D. immigrans* falls into the Drosophila s. str.

We include here the taxonomic description taken from Sturtevant's (1921) monograph on the North American species of Drosophila.

Male.—Arista with about six branches above and three below. Antennae yellow. Front over one-third width of head, wider above, yellow; ocellar dot dark brown. Second orbital one-fourth size of other two. Second oral bristle over one-half length of first. Carina broad, flat; face yellow. Proboscis yellow. Cheeks yellow; their greatest width about one-third greatest diameter of eyes. Eyes with rather thick pile.

Acrostichal hairs in eight rows; no prescutellars. Mesonotum and scutellum dull brownish-yellow. Pleurae and legs pale yellow. Apical and preapical bristles on first and second tibiae, preapicals on third. A row of very short, stout bristles on lower apical part of first femur. Basal joint of first tarsus about half as long as corresponding joint of middle leg, and thicker. Second tarsal joint of first leg also somewhat thickened and shortened.

Abdomen dull yellow, each of the four basal segments with an interrupted posterior black band. The band on the fourth segment is sometimes entire. Fifth segment black.

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A single bristle at tip of first costal section (before distal break). Wings clouded at tips of first and second veins and on posterior cross-vein. Costal index about 4.4; fourth-vein index about 1.2; 5x index about 1.0; 4c index about 0.5.

Length body 2.5 mm.; wing 2.7 mm.

Female.—Same as above, except basal tarsal joint of first leg about two-thirds as long as corresponding joint of second leg, not thicker. Second joint of tarsus of first leg not shortened or thickened.

GEOGRAPHICAL DISTRIBUTION AND ECOLOGY

Sturtevant (1921) gives many collection records from widely separated regions of the United States and from Costa Rica, Norway, Australia, and the Hawaiian Islands. The same author (Sturtevant, 1927) lists specimens from Formosa and India, and describes a new variety, *immigrans formosana.* Duda (1924) records it from Europe and Formosa, and Kikkawa and Peng (1939) from Japan. Sturtevant comments on its rarity in early collections, the first American specimens being taken in 1913. He thinks that it may be of Pacific origin. The presence of several closely similar forms in the Philippines and other parts of the Far East lends weight to this view. Today the species is very common throughout the United States. I have taken it in Massachusetts, New York, New Jersey, Pennsylvania, Tennessee, Ohio, Missouri, and California. It represents a highly successful introduced form in many sections and local communities of the United States.

Drosophila immigrans is more tolerant of low temperatures than a number of tropical species which form large summer populations in the northern part of the United States. *Drosophila hydei, melanogaster* and *simulans* are always completely killed off outdoors in this latitude, and overwinter only in buildings. Immigrans, on the contrary, is capable of surviving the milder winters outdoors, but is killed off by a severe season. This fact has been well established for northern Ohio through collections made over a period of years by Harrison Stalker and the author. In some years immigrans is rare or absent from fairly extensive spring collections, but even in these years it can be taken in abundance in the autumn. In other years, the summer of 1939 for example, following a mild winter immigrans appeared in numbers in collections of early summer from woods as well as in town.

It appears that *Drosophila immigrans* can breed in the woods, possibly on the same type of food as robusta, affinis, and

other non-fungus feeding woods species. However, it would seem to be more successful in town, breeding on over-ripe fruit, garbage, etc. Following a cold winter the few individuals which over-winter indoors give rise to a small spring population which gradually spreads, building up where food, temperature, and humidity are favorable and in many cases reaching woodland territory. If the following winter is mild flies over-winter in the woods, probably among leaves, under logs and rotting stumps, and in similar sheltered spots. These flies are then ready to build up a large spring population which will be represented in early summer collections from these localities. I have placed cultures of *Drosophila immigrans* outdoors in the autumn under air temperatures considerably below freezing, and have found that some of the flies survive after a two-day exposure.

Drosophila immigrans has a rapid life cycle, fourteen days from egg to egg under optimum conditions. These conditions are probably seldom reached under natural environments, particularly on account of the fall in night temperature even in summer to a point below that at which larvae grow most rapidly. However, this species has the shortest life cycle of the larger Drosophila. This fact, coupled with a high fecundity under favorable conditions, makes possible the building up of large populations within a short time in a given locality. Where seasonal fluctuations in temperature and moisture are limiting factors, a few days difference in length of life cycle has an important bearing on the flaring out of populations from small foci, the time at which an approximate breeding equilibrium may be reached, and the degree of homogeniety of local colonies which have bred up to a point where all available food is being used.

Drosophila immigrans, breeding in the northern United States, is a species which in mild years is likely to form spring and early summer populations large in area but low in density, breeding in the woods as well as in towns, on food similar to that used by typical native woods species. The dense localized populations developing later in the summer on accumulations of refuse, decaying fruit, etc., are likely to have sprung from migrants working in from many surrounding sources. In contrast, after a hard winter there will be no widespread spring and early summer populations and the dense aggregates formed in late summer and early autumn will be more spotty in distribution and homogeneous in structure, due to their development

from small localized foci of flies over-wintering indoors or in particularly favorable locations for winter survival. The migration out into woodland territory in such a season will progress into the autmun providing the proper humidity conditions are present.

A study of Drosophila populations breeding on a large citrus dump near Azusa, Southern California, and on a refuse heap from a canning factory and fruit and grocery wholesaler near Wooster, Ohio, has given some data on the breeding habits of the species present. In both places *Drosophila hydei* is the dominant form. Immigrans, melanogaster, simulans, and buskii are found in smaller numbers. Others also occur but in still less abundance. In both situations almost pure stands of hydei in all stages of development may be found in many parts of the breeding ground. However, small areas may be found where immigrans or melanogaster predominate in collections of larvae, pupae, and adults. An explanation of this localized concentration of minorities in a habitat where one species is vastly in the majority depends on a knowledge of such factors as breeding habits, micro-environments, and the actual sequence of events necessary to ensure the inception and continued development of a given generation of a species. Actually, both in California and Ohio the flies seek shelter from the direct rays of the hot, mid-day sun. Then in late afternoon they come out and feed voraciously in spots where the humidity is high and the food supply abundant and of the right quality. This occurs at relatively high temperatures. As the temperature continues to drop in the late afternoon courting and copulation occur. At a still lower temperature oviposition takes place in humid areas which are likely to be protected from rapid drying. A favorite place for egg-laying is a crevice in relatively fresh food. There is often some desultory inter-specific courting, but the tendency is for groups of one species to congregate in a limited area. This is followed by intra-specific courting and copulation. It seems likely, although the observation has not been made, that the females remain close to the point of copulation and deposit their eggs in the immediate vicinity later in the evening. The output from a few dozen females would be sufficient to account for these small local populations of a minority species observed here and there in the general habitat. Whether there is some local favorable stimulus which draws a minority species together at a given point I have been unable to determine. It may only

be that the mutual attraction of individuals of a given species tends to form concentrated local groups at copulation and egg-laying time. One gets the impression that both immigrans and melanogaster populations are breeding on fresher food than hydei (Spencer, 1932)

There is no doubt but that adults, pupae, and larvae of immigrans as well as other species are capable of withstanding much higher temperatures outdoors than in bottle culture without sterilization or injury. This seems in large part due to the removal of deleterious gases as carbon dioxide under the better ventilation of the natural environment. Furthermore, rapid evaporation from surfaces exposed to air of low or medium humidity undoubtedly lowers the temperature in the micro-environment of the air film of a couple of millimeters thickness to which the fly is actually exposed when resting on the surface in question.

PHYSIOLOGY

A number of facts of interest concerning the life cycle and physiology of this fly have been observed, some of which have an important bearing upon its adaptability as a form for genetic study. As stated above, the life cycle under optimum conditions is fourteen days. This may be divided into egg, larval, pupal, and adult periods. The eggs are much smaller than in most Drosophila species, have four tapering filaments, and are buried deeply in the food medium when this consists of corn-meal or banana agar. They require approximately thirty hours to hatch at 24 C. Both larvae and adults are very sensitive to acidity and excess carbon dioxide formation in vial or bottle cultures. For this reason high temperatures should be avoided where fermentation is likely to occur, as in cultures where yeast is being grown. Immigrans larvae grow quite well on Fleischmann's yeast suspension, using "kleenex" tissue as a base. Flies from larvae grown on this medium are large and vigorous. Where yeast suspension is used for the larvae, adults may be fed on corn-meal molasses agar with a minimum of yeast. The use of "moldex" has made the culture of immigrans much easier, as this ingredient in the medium cuts down bacterial, mold, and yeast growth to a point where there is little danger of adult flies being injured by carbon dioxide formation at temperatures below 25 C.

Immigrans is similar to hydei and melanica and unlike virilis and funebris in requiring re-culturing for each new generation. It is not safe to add fresh food to old culture bottles of immigrans as the whole culture is likely to deteriorate from bacterial growths. With funebris or virilis the addition of fresh food to old cultures keeps them running indefinitely. The difference in species in regard to the length of time they may be kept without re-culturing seems to depend on the ability of their larvae to utilize old, worked-over media, and the tendency for certain species to be associated with bacterial growths which prove deleterious.

The species is intermediate in activity between such active forms as melanogaster, simulans, hydei, and repleta and sluggish types as funebris and robusta. It etherizes slightly faster than melanogaster and slower than robusta, which places it about the center of the range of etherization-time of the species of Drosophila investigated. It has the habit of defecating as it goes under ether. This is likely to prove inconvenient for the investigator where large numbers of immigrans are being etherized. To keep the flies clean it is advisable to use a large etherizer or to handle fewer flies at a time.

Of all the species studied the males of immigrans become sexually mature the earliest after eclosion. Within from four to six hours after emergence males have been observed copulating. Although females do not begin ovipositing for three to four days they may be impregnated soon after eclosion. The flies remain in copulation for as much as an hour in some cases. The extreme sexual precocity of the male is a serious handicap to the use of this species for extensive genetic work requiring controlled matings of diverse stocks.

CYTOLOGY

Metz (1916) first described the metaphase chromosomes as consisting of three rods and a V. Stella (1936) has corroborated this finding. This author has made a study of oogenesis, following the methods used by Guyenot and Naville, and finds that unlike melanogaster no premeiotic and meiotic stages are to be found either in young or old female pupae. Meiosis in immigrans begins after the emergence of the female and can be studied by fixing ovaries from young females. Early embryonic mitoses occur five to six hours after egg-laying.

Emmens (1937) has made a study of salivary gland nuclei in this and three other species. He finds five chromosome arms as would be expected from the three rods and a V-shaped chromosome in metaphase plates. He finds the chromocenter to be larger and more diffuse than in melanogaster and funebris. He describes an element unique in the salivary nucleus of immigrans under the name of "striated body." This consists of a wide, short element, with four or six bands. He interprets this as probably consisting of the proximal ends of several or all the chromosomes fused laterally. The suggestion is also made that this body might consist of fused trabants from several chromosomes.

TABLE I

A LIST OF MUTANTS OF *Drosophila immigrans* DESCRIBED BY VARIOUS WORKERS. Autosomal recessive marked a. r.; sex-linked recessive marked s. r.

	NAME	PHENOTYPIC EFFECT	DESCRIBED BY	DATE
1	Abnormal a. r.	Plexus veins	Bischler and Piquet	1931
2	Axillary a. r.	Venation	Metz and Metz	1915
3	Brown s. r.	Eye color	Bischler and Piquet	1931
4	Carmine opaque a. r.	Eye color	Bischler and Piquet	1931
5	Extra bristles	Dorso-centrals	Bischler and Piquet	1931
6	Ski	Upturned wings	Bischler and Piquet	1931
7	Spread a. r.	Wing position	Bischler and Piquet	1931
8	Truncated a. r.	Bristles	Bischler and Piquet	1931
9	Yellow s. r.	Body color	Stella	1936
10	..... s. r.	Small-wing	Sturtevant	1921
11	..... a. r.	Small bristles	Sturtevant	1921
12	..... a. r.	Modified veins	Sturtevant	1921
13	..... a. r.	Modified veins	Sturtevant	1921
14	..... a. r.	Modified veins	Sturtevant	1921

GENETICS, SUMMARY OF DESCRIBED MUTANTS

In Table I is given a summary of the data on mutants published up to the present time in this species. Of the fourteen mutants described, nine were autosomal recessives, three sex-linked recessives and two not analyzed. Of these only yellow body, sex-linked recessive reported by Stella (1936), is clearly a parallel of mutants found in other species, although the others might well be parallels as they are similar to mutants known for other flies. The abnormal venation of Bischler and Piquet (1931) and possibly two of the venation mutants reported by Sturtevant (1921) may be alleles at the "net" locus (see below).

GENETICS, NEW MUTANTS

In Table II is given a summary of new mutants, hitherto unpublished. These were found by the author, except for three discovered by Sturtevant and turned over to the author. Sturtevant has also found several other cases of "net," which are probably alleles of those reported here. There follows a brief description of each of the mutants listed in Table II. A number of these have been described briefly in Drosophila Information Service, No. 11, and there assigned a symbol. It is not deemed advisable to publish symbols here as the stocks have been discarded. Consequently anyone in future making a genetic study of this species will not have the confusing situation of avoiding the use of symbols assigned to mutants no longer extant in laboratory stocks. The descriptions are necessarily brief, but it is hoped that they have been made sufficiently specific and clear that they may be used for comparative purposes. Of the thirty-seven mutants described all but four were kept in stock for several generations, either in pure form or through the mating of heterozygotes when sterility was involved. The four not carried in stock were distinct enough in phenotypic effect and appeared in sufficient numbers in F_2 cultures from wild flies to make certain that they were true mutant types and not aberrant forms due to some developmental "accident." Many of the stocks were carried for two years. They have been discarded through the press of other work and the conclusion that the detailed study of linkage in the species was not advisable, owing to the technical difficulty referred to above.

Cherry. Sex-linked recessive. A translucent eye color found in one male of a stock derived from wild flies collected at Gatlinburg, Tennessee. It probably arose as a laboratory mutant not present in the original wild material. Classification easy and viability good.

Singed. Sex-linked recessive. Mutant causing a singing of all bristles and hairs, including marginal bristles of wing. Several singed males were found in a mass culture descended from a pair of wild flies collected in Wooster, Ohio. Females were sterile, males with fair viability and fertility. This seems a clear parallel of singed in melanogaster and other species, and represents a medium to extreme allele.

Brown. Autosomal recessive. A dark eye color, easily classified at eclosion and darkening with age to almost black. The original name given in DIS 3 is retained, although this is more like weak sepia or clot of melanogaster. Found as one female in mass culture from stock of wild flies collected in Wooster.

Burnt. Autosomal recessive. A peculiar mosaic eye color from wild stock, Azusa 17. This and almost all of the remaining mutants

described appeared as several flies segregating out of F_2 mass cultures reared from females either impregnated before capture or mated to a single wild male taken at the same time. The burnt character consisted

TABLE II

A LIST OF *Drosophila immigrans* MUTANTS DESCRIBED IN THIS PAPER

	NAME	PHENOTYPIC EFFECT	FROM WILD STOCK
Sex-linked Recessives			
15	Cherry	Eye color	Gatlinburg
16	Singed	Bristles	Wooster
Autosomal Recessives			
17	Brown	Eye color	Wooster
18	Burnt	Eye color	Azusa, 17
19	Curly	Wing shape	Azusa, 24
20	Dark	Eye color	Azusa, 8
21	Extra	Venation	Wooster
22	Extra dorso-centrals.	Bristles	Woods Hole
23	Extra scutellars	Bristles	Azusa
24	Forked scutellars	Bristles	Azusa, 30
25	Grooveless	Scutellar groove	Azusa, 14
26	Irregular	Hairs, wings	Azusa, 1
27	Irregular	Hairs, wings	Azusa, 8
28	Javelin	Bristles	Arroyo Seco
29	Minute	Bristles	Rincon, 17
30	Minute mosaic	Bristles	Azusa, 5
31	Net	Venation	Azusa, 13
32	Net	Venation	Azusa, 24
33	Net	Venation	Azusa, 29
34	Net	Venation	Rincon, 5
35	Net	Venation	Rincon, 14
36	Net	Venation	Gatlinburg
37	Net	Venation	Gatlinburg
38	Net	Venation	Woods Hole
39	Ocelliless	Bristles, ocelli	Azusa, 3
40	Peach	Eye color	Azusa, 5
41	Peach-like	Eye color	Azusa
42	Rough	Eye texture, veins	Azusa, 5
43	Rough-like	Eye texture, veins	Azusa, 17
44	Rough-like	Eye texture, veins	Rincon, 8
45	Slight dark	Eye color	Rincon, 2
46	Slight dark	Eye color	Wooster, 3
47	Small eye	Eye shape, texture	Rincon, 18
48	Stubby	Bristles	Woods Hole, 43
49	Stubby-like	Bristles	Gatlinburg
50	Two bristle	Bristles	Azusa, 10
51	Two bristle	Bristles	Woods Hole, 69

NOTE.—Javelin, net Azusa 13, and peach-like found by A. H. Sturtevant and turned over to the author for study. All other mutants listed here were found in 1937 except brown (1934), cherry (1938), extra (1933), two Gatlinburg nets and stubby-like (1938).

of a dark area of variable size, located near the center of the eye if small; sometimes covering most of the eye. Young, unetherized flies failed to show this. When etherized the burnt area rapidly appeared,

and did not again fade out. Old flies without etherization developed this dark area. Frequently the area had a shiny, seared appearance. The mutant also colored the malpighian tubules a bright salmon. Burnt was somewhat infertile and showed great variability in expression, but with few normal overlaps.

Curly. Autosomal recessive. Wings curled upward as in the dominant Curly of melanogaster. A mutant of very poor viability, and partially sterile. Owing to sterility of females a pure stock was never established. From Azusa 24.

Dark. Autosomal recessive. A dull, dark eye color, somewhat variable and difficult to classify in some cultures. From Azusa 8.

Extra. Autosomal recessive. A slight venation mutant, consisting of small cross-vein or several of these near the distal terminus of the second longitudinal. Normal overlaps. From wild stock collected in Wooster.

Extra dorso-centrals. Autosomal recessive. Two or more extra dorsocentral bristles. Variable in expression. This character is frequently met with in wild collections of immigrans but segregated rather definitely in certain wild strains collected from Woods Hole, Massachusetts, while not appearing in others and is consequently listed as a mutant.

Extra scutellars. Autosomal recessive. Extra anterior scutellar bristles of about equal size to the normal ones and lying close to them. Very inconstant in expression. From wild stock of Azusa flies.

Forked scutellars. Autosomal recessive. Anterior scutellars gnarled or forked. Often poorly expressed with many normal overlaps. From Azusa 30.

Grooveless. Autosomal recessive. Similar to the fourth chromosome mutant of this name in melanogaster. When first found the character was well expressed, with the entire lack of a groove between scutellum and thorax. However, there were no dark excrescences on thorax as in melanogaster and hydei. After several generations the expression became less marked with normal overlaps. Since grooveless was recovered from outcrosses modifiers may have accumulated. From Azusa 14.

Irregular. Autosomal recessive. Bristles along margin of wing standing out at a wide angle; hairs on abdominal tergites disarranged; hairs on thorax disarranged; wings somewhat shorter than normal and held out at an angle. Similar to mutant by same name described by Chino (1929) in Drosophila virilis, and found by the author in hydei, melanogaster, robusta, and an undescribed species in the repleta group (all unpublished). This seems to represent an extreme type by comparison with the others. From Azusa 1.

Irregular. Autosomal recessive. Bristles along margin of wing standing out at wide angle; hairs of abdominal tergites slightly disarranged, but thoracic hairs normal and wings only slightly shortened. A slight allele as shown by mating tests with extreme irregular. From Azusa 8.

Javelin. Autosomal recessive. Mutant found by A. H. Sturtevant in wild stock from the Arroyo Seco, a canyon in the San Gabriel mountains near Pasadena. Bristles long, not tapering at the end as in wild-type; often hooked at end. Similar to mutant of same name in melanogaster.

Minute. Autosomal recessive. Very small bristles; low viability and fertility; pure stock not established. From Rincon 17.

Minute mosaic. Autosomal recessive. Small bristles in a mosaic pattern; anterior scutellars most often affected; less frequently dorsocentrals and head bristles; pattern seemed to show no symmetry. From Azusa 5.

Net. Autosomal recessive. Found by A. H. Sturtevant in his stock Azusa 13, from a single female collected near Azusa. This was a very extreme net venation with a heavy plexus over most of the surface of the wing, and particularly concentrated around the posterior cross-vein and along margin of wing between first and second longitudinals. Although there was much variability in a culture all flies showed the character in extreme form. This mutant in some cases showed weak net manifestation in heterozygous form when crossed to stock showing no visible net, indicating that extreme net, Azusa 13, is partially dominant to some phenotypically wild-type alleles.

Net. Autosomal recessive. This and the following cases of net seem to represent various alleles at the frequently mutating net locus in this species. While tests for allelism were not made in all combinations, in cases where they were made the results were consistent with the view that there was a large series of multiple alleles at the net locus, distributed through wild populations. This is a situation somewhat similar to the case of the multiple allelic series of "bobbed" in Drosophila hydei (Spencer, 1937). This was a net of medium grade of expression, with plexus of veins developed around posterior cross-vein. From Azusa 24.

Net. Autosomal recessive. Weak expression, but no normal overlaps. Small sections of vein around posterior cross-vein and between first and second longitudinals. From Azusa 29.

Net. Autosomal recessive. Medium expression. From Rincon 5.

Net. Autosomal recessive. Medium to weak expression. From Rincon 14.

Net. Autosomal recessive. Medium to weak. From Gatlinburg.

Net. Autosomal recessive. Weak expression. From Gatlinburg.

Net. Autosomal recessive. Very weak expression. Recovered in ten cases from tests of 156 wild flies from Woods Hole. In appearance these all seemed very similar and might readily have come from a single source (see discussion below).

Ocelliless. Autosomal recessive. Similar to mutant of the same name described by the author in Drosophila funebris (Spencer, 1928). Hairs sparse on thorax and abdomen, wing marginal bristles ragged; ocellar bristles sometimes missing; ocelli may be missing or run together; eye pile sparse; wings thin textured; sometimes hairs or bristles doubled and coming from same basal ring; partially sterile and inviable. From Azusa 3.

Peach. Autosomal recessive. Translucent eye color when newly emerged; darkening with age. From Azusa 5.

Peach-like. Autosomal recessive. Translucent eye color, somewhat easier to classify than peach. Found by A. H. Sturtevant in Azusa wild stock.

Rough. Autosomal recessive. Extreme rough eye; venation ragged and wings thin textured. Semi-sterile. From Azusa 5.

Rough-like. Autosomal recessive. Similar to rough of Azusa 5, but less extreme. Allelism not tested due to difficulty of breeding rough. From Azusa 17.

Rough-like. Autosomal recessive. Almost identical to rough of Azusa 5, but allelism not tested. From Rincon 8.

Slight dark. Autosomal recessive. Dark eye; not as extreme as brown and frequently difficult to classify. From Rincon 2.

Slight dark. Autosomal recessive. Phenotypically similar to slight dark from Rincon 2; allelism not tested. From Wooster 3.

Small eye. Autosomal recessive. Small, rough eye with anterior scutellars missing on most flies. Inviable and sterile. From Rincon 18.

Stubby. Autosomal recessive. All head and thoracic bristles short and thick. Viability and fertility good, and classification easy. From Woods Hole 43.

Stubby-like. Autosomal recessive. Similar to stubby of Woods Hole 43. Allelism not tested. From Gatlinburg.

Two bristle. Autosomal recessive. Anterior dorso-central bristles missing. Good expression, but with a few normal overlaps. The species often gives expression to extra dorso-centrals. This mutant has a phenotypic effect opposite to the general trend of the species. From Azusa 10.

Two bristle. Autosomal recessive. A mutant similar to the one just described but with many more normal overlaps. From Woods Hole 69.

In addition to the above mutants the author has recorded several cases of weakly expressed and inconstant hereditary types. In any study of visible mutants it becomes obvious that there are many grades ranging from those which under any environmental conditions which will allow for the development of the flies show a constant and uniform character expression to those inherited tendencies which give no visible effects under some conditions and only feeble and inconstant characters under other conditions. In spite of this range of character expression it is possible to list in a given investigation those mutants which show about the degrees of expression at a phenotypic level generally worked with by students of Drosophila genetics. The argument is sometimes advanced that lethal genes can be handled more objectively. However, to those familiar with the gradations from what appear to be 100% recessive lethals, through semi-lethals which under slightly adverse culture conditions show 100% lethal expression, to mutants of low viability, this argument should have little weight. The accuracy of the comparison and contrast of data collected from different sources, as in the study of diverse populations reported here, will depend less on where the line

is drawn as regards classifying of attenuated phenotypic effects as mutant types, than on drawing that line at about the same point for all sets of data under consideration. Furthermore, a thorough familiarity with the peculiarities exhibited by a species under various conditions of culture, against a background of experience gained through the study of other species, is probably more important than the setting up of arbitrary rules of classification to be followed blindly. While it may appear to be a dangerous practice it is none the less important that an investigator be prepared to evaluate results from a particular experiment in terms of all that he knows of possible factors involved in giving the results.

At best, however, it is impossible to carry on investigations which deal either with the origin of new mutants or the distribution of mutants already present in a population without some subjective error. These errors are less when the amount of data treated is large, and when the data are collected by one rather than by several workers.

LINKAGE STUDIES

The X-Chromosome. The sex-linked recessives, brown eyes (Bischler and Piquet, 1931), cherry eyes (Spencer), small wing (Sturtevant, 1921), singed bristles (Spencer), and yellow body (Stella, 1936) have been recorded. However, these mutants were found at various times in widely separated laboratories and no linkage studies on the X-chromosome have been carried out. Singed and yellow are clearly parallels of mutants found in the X of many other species, and the others might well be parallels, although their identity is not so readily determined.

Autosomal Linkage. Bischler and Piquet (1931) report linkage between spread wings and carmine opaque eyes.

Sturtevant (unpublished) found that net and brown were linked.

I have added to this linkage group the gene rough, Azusa 5, and find that peach, Azusa 5, and javelin are in the same linkage group and not linked to brown-net-rough.

Thus three of the four expected linkage groups have been established from the investigation of a few of the mutants reported here. The author made preliminary attempts to secure further linkage data, but these were unsuccessful owing to the difficulty of securing virgin females for tests, as noted above.

No cross-over data are available, and the genetic chromosomes of this species remain wholly unmapped, Linkage studies on *Drosophila immigrans* would be possible, but could only be carried through successfully by one with much time available for the work.

GENETIC STRUCTURE OF POPULATIONS

The new mutants found by the author have been extracted from five geographically distinct populations. It is realized that the samples studied were inadequate in size and that the method of analysis was less accurate than those used by Dubinin and collaborators (1934), Gordon (1936), and the Timofeeffs-Ressovsky (1927) for the analysis of visibles in *Drosophila melanogaster* populations, or of Spencer (in press) for similar work on *Drosophila hydei.*

Wild flies were trapped at Camp Rincon, San Gabriel Canyon, Southern California; a large citrus dump near Azusa, Southern California; Gatlinburg, Tennessee; Woods Hole, Massachusetts (two stations); and a refuse dump from a canning factory, Wooster, Ohio.

The method of analysis consisted of rearing an F_1 generation either from a female impregnated before capture or from a pair of flies taken from traps and mated in the laboratory. One or two F_2 mass cultures were reared from about a dozen of the F_1 flies. Neither lethals nor chromosomal variations were studied. If random mating occurred among the F_1 flies, then approximately one-sixteenth of the F_2 flies should be homozygous for a given recessive autosomal mutant carried in heterozygous form by one of the original parents. The inaccuracy of the method consists in part in the practical certainty that the F_2 flies are not produced at random from all the F_1 parents used. This has been proved when the method of rearing the F_2 from several separate pair matings is directly compared to the F_2 mass culture method. However, as the same technique was used throughout these analyses are directly comparable.

Table III summarizes the results from the several populations as to the number of flies tested from each region and the number of separable visible mutants or alleles of these recovered. In this table the chi square test has been applied to determine whether the distribution of visible mutants recovered is random for the five populations studied. By this test it is found that the distribution is far from random, the deviations

being highly significant. A much larger group of mutants was discovered for the size of the sample taken from Azusa than for the sample from Woods Hole. The Wooster and Rincon populations show small deviations from expectation, while the Gatlinburg population contained very nearly the same proportion of mutants as that found for all the samples together.

These facts may be explained by reference to the pattern of the populations in question. At Woods Hole the species is probably killed off outdoors in the winter. The Woods Hole collections taken at two trapping stations about one-half mile

TABLE III

SUMMARY OF WILD FLIES TESTED AND AUTOSOMAL RECESSIVE MUTANTS RECOVERED FROM FIVE GEOGRAPHICALLY DISTINCT POPULATIONS OF *Drosophila immigrans* AND CALCULATION OF CHI SQUARE AS A TEST OF THEIR RANDOM DISTRIBUTION

LOCALITY OF POPULATION	TOTAL NUMBER WILD FLIES TESTED, n	NUMBER OF DISTINCT MUTANTS RECOVERED, X	NUMBER OF MUTANTS EXPECTED m	$\frac{362}{330}\cdot\frac{(X-m)^2}{m}$
Azusa, California	60	16	5.304	23.661
Camp Rincon, California	56	6	4.950	0.245
Gatlinburg, Tennessee	44	4	3.890	0.003
Woods Hole, Massachusetts	156	4	13.790	7.624
Wooster, Ohio	46	2	4.066	1.152
Totals	362	32	32.000	32.69 = χ^2 d. f. = 4

Probability of chi square being 13.277 or greater is 0.01; as chi square is actually 32.69 the deviation from a chance or random distribution of distinct mutants in the five populations is such as would happen much less often than once in 100 trials and is clearly significant.

apart in late July came in all probability from small local foci which had overwintered indoors. The fact that a net mutant of mild expression was found ten times in the sample of 156 flies tested is indicative of this. Two other mutants were also found several times. In all only four distinct types were recovered. In contrast sixteen distinct mutants were extracted from 60 flies tested from Azusa. Here the species survives the year round outdoors, although the population must be markedly decreased during the hot, dry summer. The effective breeding population at the Azusa citrus dump is quite conceivably much larger than that of Woods Hole, where the breeding aggregate

contracts yearly into an indoors "bottle-neck." These data are in line with the mathematical analysis of Wright (1931) on the breeding structure of Mendelian populations. The figures are small and the analysis somewhat faulty. However, the evidence presented here indicates the importance of the size of populations at different times of the year in determining their genetic structure.

SUMMARY

1. The taxonomic position of *Drosophila immigrans* is reviewed.

2. Known facts of its distribution in Europe, Asia, and North America are given.

3. Collection data show that in mild seasons the species may over-winter outdoors in northern Ohio; in severe winters it survives only in buildings. This affects the structure of the summer and autumn populations in a given season.

4. Immigrans forms small, local aggregates in breeding grounds where *Drosophila hydei* is the dominant form.

5. The species tolerates higher temperatures outdoors than in bottle cultures, probably due to better ventilation.

6. Larvae and adults are very sensitive to acidity and to accumulation of carbon dioxide. Larvae grow well on a "kleenex" yeast suspension medium.

7. Males show extreme sexual precocity, making controlled matings of diverse stocks difficult.

8. The cytology is reviewed briefly. There are three pairs of rods and one pair of V-shaped chromosomes. The salivary chromosomes show five long elements.

9. A summarized table of 14 mutants from descriptions in the literature is given.

10. A table and brief descriptions of 37 new mutant types are presented.

11. Of these a multiple allelic series at the "net" locus is most interesting. Evidence presented indicates that net is widely spread throughout immigrans populations.

12. Sex-linked yellow and singed are clear parallels of mutants of the same names in other species. Other parallels are not so evident.

13. In Table III appears a summary of the genetic analysis for recessive visible mutants present in 362 wild flies from populations at Camp Rincon and Azusa, Southern California; Gatlinburg, Tennessee; Woods Hole, Massachusetts; and Wooster, Ohio.

14. The chi-squared test shows that the mutants recovered were not distributed at random to these five populations.

15. The excess of mutants at Azusa and their scarcity at Woods Hole are interpreted in terms of the breeding structures of the populations involved.

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